Biochemical and Biophysical Research Communications **268,** 106–111 (2000)
doi:10.1006/bbrc.1999.2061, available online at http://www.idealibrary.com on IDEAL®

Peptide Mimicking Sialyl-Lewis[a] with Anti-inflammatory Activity

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Received December 22, 1999

Peptides mimicking carbohydrate structure sialyl-Lewis a (SA-Le[a]) have been selected from a diverse dodecapeptide library using monoclonal antibody (MAb) NS19-9. Families of peptides with a consensus sequence consisting of three to nine amino acids and peptides that do not show a conserved core amino acid region were identified. Peptide DLWDWVVGKPAG was selected based on the consensus sequence DXX-DXXVG shared with other peptides and strong binding in Western blot. Peptide competes with antibody binding to its native carbohydrate antigen, SA-Le[a], at 50% inhibitory concentration (IC_{50}), 700 μM, implying that it represents a structural mimic of the carbohydrate epitope recognized by MAb. Statistically significant reduction of neutrophil recruitment into the intraperitoneal cavity was observed upon administration of this peptide in a murine acute inflammation model *in vivo*. Results suggest that the peptide mimic of SA-Le[a] carbohydrate might bind to E-selectin and block its interaction with another ligand, sialyl-Lewis X (SA-LeX), expressed on neutrophils. © 2000 Academic Press

Functional equivalence of the chemically dissimilar molecules such as carbohydrates and proteins sharing common surface topology have been identified previously due to the combinatorial technologies available in recent years as well as a naturally occurring phenomena. Although cross-reactive peptides have been described for several anti-carbohydrate antibodies and lectins (1–10) the recognition of carbohydrate structures and their mimics may differ. While the cross-reactive peptides were found for most if not all anti-carbohydrate antibodies, only few of many studied lectins have been shown to bind peptides (4–6). Peptide mimetics of carbohydrate ligand for concanavalin A (Con A) methyl α-D-mannopyranoside (5, 6) and a ligand for *Ricinus communis* lectin β-D-galactose have been identified (7). Furthermore, the peptide mimicking α-D-mannopyranoside was recognized by anti-carbohydrate antibodies and Con A as well as anti-peptide antibody (4). Similarly, antibodies induced against peptide mimetics of hexasaccharide carcinoma associated antigen, Lewis Y (LeY) were cross-reactive with a cognate antigen (9, 10). These data suggested that peptides are true molecular mimic of carbohydrate determinants.

The naturally occurring mimicry between proteins and glycan residues has also been identified. Tendamistat, a regulatory protein inhibitor of α-amylase, binds to the carbohydrate-binding site of the enzyme through a tripeptide epitope (11). Polysaccharides of Streptococcal group A bacteria and host gangliosides induce antibodies cross-reactive with proteins, leading to the clinical manifestations of autoimmune responses (12–14). These examples suggest that carbohydrate mimics can be effective in manipulating structurally and functionally important protein carbohydrate interactions and that it is possible to isolate molecules binding receptors without being chemically similar but rather being structurally equivalent.

Selectins are tumor necrosis factor α and interleukin 1β inducible, calcium-dependent molecules, which are expressed on vascular endothelium during the early stage of inflammatory reaction (15, 16). A multistep process initiated by the selectin family enables neutrophil extravasation as reviewed earlier (17). Neutrophils initially adhere to selectins, which in turn promote activation of neutrophil β2 integrins, leading to integrin-dependent firm adhesion to the integrin receptor ICAM-1, and finally to neutrophil extravasation, possibly via interaction of platelet/endothelial cell ad-

Abbreviations used: Con A, concanavalin A; ELISA, enzyme linked immunosorbent assay; HRP, horseradish peroxidase; IC_{50}, 50% inhibitory concentration; IgG, immunoglobulin; ip, intraperitoneally; iv, intravenous; MAb, monoclonal antibody; MPO, myeloperoxidase; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMN, polymorphonuclear leukocytes; SA-Le[a], sialyl-Lewis a; SA-LeX, sialyl-Lewis X; SDS, sodium dodecyl sulfate.

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0006-291X/00 $35.00

hesion molecule 1. Integrin-FAK-Src pathway in transducing the recognition signal from outside to inside the cell is postulated during this process (18).

Significant progress has been made in the understanding of the role of oligosaccharides in inflammation. The initial interaction is mediated by the adhesion of neutrophils to endothelial cells E- and P-selectins through the multivalent interaction of glycoconjugates carrying the terminal tetrasaccharide sialyl-Lewis X (SA-LeX), [NeuAcα 2,3Gαlβ1,4(Fucα1,3) GlcNAcβ1,3Galβ1,4Glcβ1-R] on the cell surface (19). E-selectin also binds with slightly higher affinity a structural analog of SA-LeX, sialyl-Lewis a (SA-Le^a) [NeuAcα2,3Galβ1,3(Fucα1,4) GlcNAcβ1,3Galβ1,4Glcβ1-R]. SA-Le^a that is not found on neutrophils and monocytes, and thus does not play a physiological role in their E-selectin-dependent adhesion. However, SA-Le^a is often found on various carcinomas which frequently also express SA-LeX (20, 21). Computer generated molecular models indicated that in the SA-LeX and SA-Le^a molecules sialic acid and fucose residues are in virtually identical positions in space providing similar topographies on each molecules for E-selectin recognition (22).

Understanding of the mechanism of carbohydrate recognition my lead to the developments of a new concepts and new strategies to design agents blocking this interaction. This strategy may be useful to treat acute inflammatory reactions such as asthma, myocardial infarction, lung injury, and arthritis. *In vivo* blockage of selectin-dependent neutrophil adhesion has been considered as an anti-inflammatory approach by many laboratories. Reagents such as monoclonal antibodies (MAbs), derivatives of SA-LeX, E-selectin binding peptides, peptides derived from E-selectin binding site and polyanions designed to block an E-selectin-carbohydrate ligand interaction have been shown effective to inhibit the cell adhesion to endothelium and to prevent deleterious inflammatory reactions (23–31). In our study, the administration of monovalent peptide mimicking one of the carbohydrate ligands for E-selectin, SA-Le^a (32) specifically reduced the neutrophil recruitment *in vivo.* This observation suggests that the interaction of E-selectin with SA-LeX on neutrophils can be blocked using mimic of an alternative ligand, SA-Le^a that is not expressed on neutrophils. Thus, such a strategy targets adhesion molecule binding site regardless of the natural ligand and can be applied to treat conditions, which involve cells expressing various carbohydrate ligands such as inflammation and metastasis.

MATERIALS AND METHODS

Antibodies and peptides. MAb NS19-9 was generated at the Wistar Institute and its specificity was previously characterized (21). Peptides were synthesized by standard solid-phase strategies and high-pressure liquid chromatography-purified at the Peptide Synthesis Facility of the Wistar Institute or by Research Genetics (Huntsville, AL). The identity of peptide structures was confirmed by fast-atom bombardment mass spectrometry at the Wistar Institute Protein Sequencing Facility. Synthetic multivalent SA-Le^a-PAA conjugated to polyacrylamide matrix was purchased from Glycotech, Inc. (Rockville, MD).

Random peptide library and library screening. In the FLITRX library (Invitrogen, Carlsbad, CA) random peptides are displayed as a fusion protein on the bacterial cell surface as conformationally constrained insertions into thioredoxin (trxA) (33). An aliquot of the library containing at least 2×10^{10} cells to ensure full representation of peptides, was grown to saturation for 15 h in IMC/amp100 medium (M9 medium containing 1 mM $MgCl_2$ supplemented with 0.5% glucose, 0.2% casamino acids and 100 μg/ml ampicillin) followed by 6 h incubation in medium containing 100 μg/ml tryptophan. The induced bacteria were panned on an MAb-coated tissue culture plate (20 μg/ml) followed by blocking with 1% nonfat milk containing 150 mM NaCl and 1% α-methyl mannoside for 1 h. The bound cells were eluted by rinsing the plate with 10 ml of fresh IMC/amp100 medium. The entire selection process was repeated four more times. Isolated bacteria were grown on ampicillin-containing plates and individual colonies were grown as a small-scale culture and analyzed using Western blot.

Western blot. The bacterial colonies isolated upon the final selection cycle were tested for protein expression using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and Western blot (34). Expression of the FLITRX fusion protein containing peptide sequences mimicking carbohydrate was detected with SA-Le^a specific NS19-9 MAb (10 μg/ml). The expression of thioredoxin on the nitrocellulose filter was confirmed after incubation with trxA-specific MAb (anti-Thio) (Invitrogen). Following antibody binding the filters were incubated with horseradish peroxidase (HRP)-conjugated goat anti-mouse immunoglobulin (IgG) (Boehringer-Mannheim, Indianapolis, IN). Finally, filters were exposed to luminol and oxidizing solution (1:1) for 1 min, air-dried and autoradiographed on Reflection film (NEN, Life Sciences Products, Boston, MA).

DNA sequencing. DNA was isolated from the selected bacteria using a mini-column DNA purification kit (Qiagen, Chatsworth, CA). The nucleotide sequence of the isolated DNA from the selected bacterial colonies was determined by the dideoxynucleotide chain termination method using FLITRX primers (Invitrogen) and fluorescent labeling (ABI) at the DNA facility in the Wistar Institute. The amino acid sequences of the peptides were deduced based on most common codon usage.

Competition binding assay. The peptide competition assays were performed using enzyme linked immunosorbent assay (ELISA). Ninety-six-well round-bottom microtiter plates (Nunc, Immuno II, Roskilde, Denmark) were coated overnight at 37°C with 50 μl aliquots of synthetic neoglycoprotein containing coupled multivalent carbohydrate determinant (SA-Le^a-PAA) (Glycotech, Rockville, MD) (0.25 pg/well) in a coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 3 mM NaN_3 pH 9.6). To avoid nonspecific binding the plates were incubated with 10% γ-globulin-free horse serum (Gibco-BRL, Grand Island, NY) in phosphate-buffered saline (PBS) at 37°C for 2 h. Test peptides at concentrations ranging from 10 to 5 mM were preincubated with 100 μl of MAb at 5 μg/ml diluted in 10% γ-globulin-free horse serum/PBS at 37°C. After 1 h MAb/peptide mixtures were transferred to wells precoated with a constant amount of neoglycoprotein (SA-Le^a-PAA) and allowed to bind for 1 h followed by blocking with 10% γ-globulin-free horse serum. Goat anti-mouse IgG conjugated to HRP was added to each well and incubated for 1 h at 37°C. The reaction was developed with tetramethylbenzidine dichloride (Sigma Chemical Co., St. Louis, MO) in 0.05 M phosphate-citrate buffer, pH 5.0, containing 0.03% sodium perborate (Sigma) and the developed blue color was read at 450 nm. Fifty-percent inhibitory concentration (IC_{50}) were calculated by non-linear least-squares regression to a four-parameter logistic equation.

Acute peritoneal inflammation. Mice were injected intraperitoneally (ip) with 1 ml of saline containing 0.5 mg/ml of Zymosan (Sigma) (35). After 3 h, the animals received slow intravenous (iv) injection of 0.2 ml of PBS containing 1 mg of peptide DLWDWVVGKPAG or control peptide. Mice were sacrificed 60 min after ip injection and the cells from peritoneal cavity were collected by lavage with 10 ml of ice-cold PBS containing 10 mM EDTA and counted in a hemocytometer. Myeloperoxidase (MPO) was extracted from cells by suspending cells in 0.5% hexadecyltrimethylammonium bromide (Sigma) in 50 mM potassium phosphate buffer, pH 6.0. Polytrone homogenization (1500 rpm) was applied followed by freeze-thawing 3 times and suspension was centrifuged at 4000*g* for 15 min. One-hundred microliters of the supernatant was mixed with 0.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml *o*-dianisine dihydrochloride (Sigma) and 0.0005% hydrogen peroxide (Sigma). The change in absorbance at 460 nm was measured spectrophotometrically. *In vivo* results were obtained from five experiments (5 mice in each group). Statistical analysis using a nonparametric unpaired *t* test was performed.

RESULTS

Peptide library screening and peptide sequence analysis. Selective enrichment of the bacteria expressing peptide library within a thioredoxine was performed using SA-Le[a] specific MAb NS19-9 coated tissue culture plates. The library was subjected to three selection procedures and each screening yielded three different families of peptides (I–III). The isolated clones (approximately 30 clones from each library panning) were analyzed on 12% SDS–PAGE and bacteria carrying peptides mimicking SA-Le[a] were identified by Western blot after probing with MAb NS-19-9.

DNA sequence of isolated clones that were positively identified on the Western blot was determined using peptide flanking primers derived from the thioredoxine sequence. The sequences of peptides deduced from DNA sequence and the relative intensity of the signal obtained upon MAb binding are presented in Table 1. The families I and II containing 4 and 6 peptides, respectively, demonstrate common motifs within the family, but the consensus sequences are not shared between the two groups of isolated peptides.

The first group includes three members that each contains VXXXG (Val-X-X-X-Gly) residues separated by three amino acids with additional preference for L, E, and S (Leu, Glu, and Ser, respectively) in the positions of intervening amino acids. Two members of this group (#2 and 3) demonstrated the presence of extended homologous sequence GXWXXVXEG (Gly-X-Trp-X-X-Val-X-Glu-Gly) spanning 9 amino acids. The sequence analysis of the peptides in this group also suggests importance of valine residue that is shared between all peptides. However, the strong signal in Western blot upon probing with MAb NS19-9 was observed only for bacterial clones expressing peptides #2 and 3 whereas, the binding of MAb to peptides expressed by clones #15 and 18 was weak. This suggests that MAb recognition correlated with in the consensus sequence GXWXXVXEG shared between the peptides #2 and 3.

TABLE 1

Amino Acid Sequences Deduced from the DNA Sequences of Bacterial Clones Isolated from the Random Peptide Using NS19-9 MAb

	Clone #	Peptide sequence[a]	MAb recognition[b]
I	#2	VGIWSVVSEGSR	+++
	#3	QDGVWEHVLEGG	++
	#15	VELSGRGGLCTW	+
	#18	TIEPVLAEMFMG	+
II	#1	RCSVGVPFTMES	+
	#4	DLWDWVVGKPAG	+++++
	#12	VIGAASHDEDVD	++++
	#14	DKETFELGLFDR	+
	#15	FSGVRGVYESRT	++++
	#19	PDDAPMHSTRVE	+
III	#1	STGLMVDFLEPG	+
	#2	AKTFGLEHGCEA	+
	#7	GGTVEVWSIKGG	+
	#9	DHFSQAGSSNHH	+
	#11	DDPVTPVIDFGK	+
	#12	RDGLIDFVVAGT	+
	#20	AIEAPQHNAGNG	++

[a] The amino acids matching the consensus binding motifs are in bold.

[b] The MAb binding is expressed as a relative intensity of signal strength visualized by Western blot immunostained with NS19-9 MAb.

Sequence alignment demonstrated that the peptides in the family II bear some positional resemblance although they do not share highly conservative consensus sequence. Two members of this group (#1 and 4) contain sequence VGXP (Val-Gly-Val/Lys-Pro). Peptide #15 contains VRGV sequence that is also partially represented within most of the peptides and shares VXG motif with peptide #4. In addition, three peptides (#4, 12, and 14) show preference for Asp and Val in similar location resulting in the motif DXXDXXVG (Asp-X-X-Asp-X-X-Val-Gly). Peptide #19 did not show amino acid sequence homology with other members of this group. Although, clones #4, 12, and 15 demonstrated the strongest binding affinity to MAb based on the signal intensity in Western blot, they display very little similarity with respect to amino acid sequence. Peptides #4 and 12 have in common DXXV motif and peptides #4 and 15 contain VXG in the middle of their sequence. Valine shared by these peptides may play a pivotal role in the binding of the peptides to MA NS19-9, although conformational constrain imposed by additional residues which differ between the peptides is needed to generate full binding energy.

The third group of peptides showed no obvious consensus sequence, but some positional homologies between individual peptides could be identified. Two amino acid motifs are shared between some of the peptides (#1 and 2; #9, 12, and 20) and glycine is found in all peptide sequences (Table 1). Bacterial clones

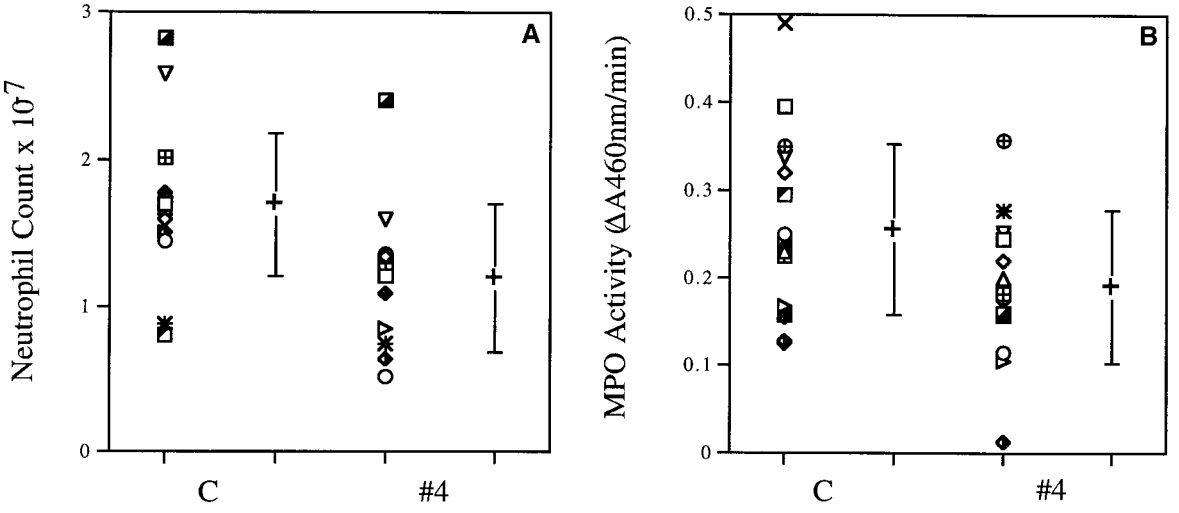

FIG. 1. Neutrophil influx upon administration of peptide #4, DLWDWVVGKPAG mimicking SA-Le[a] carbohydrate in mice with chemically induced peritonitis. Mice were injected ip with mg of Zymosan. After 3 h, animals received iv injection of 1 mg of peptide #4 or control peptide. Mice were sacrificed 60 min after iv injection and the PMN number (A) and MPO activity (B) in the peritoneal lavage was evaluated. Statistical analysis using nonparametric unpaired *t* test gave *P* values <0.023 and <0.043 for data in A and B, respectively. Values for each mice are depicted with different symbol.

expressing the peptide sequences identified in this group showed weak binding except for #20, which generated relatively stronger signal with NS19-9 MAb in Western blot. These peptides do not share any sequence homology with the peptides selected in groups I and II.

Competition of MAb NS19-9 binding to carbohydrate antigen with synthetic peptide. The peptide #4 from the family II, DLWDWVVGKPAG was selected to evaluate interaction with NS19-9 MAb, because it contained a consensus motif partially present in multiple clones after panning with NS19-9 MAb. Moreover, bacterial clone expressing this peptide displayed strongest signal in Western blot implying that the peptide may have the highest binding affinity with the MAb compared to the other identified peptides. The ability of the peptide DLWDWVVGKPAG to mimic SA-Le[a] was tested by competition of the peptide with the cognate oligosaccharide determinant for MAb binding using ELISA. Synthetic neoglycoprotein containing SA-Le[a] was immobilized on the ELISA plate and MAb NS19-9 was bound to this antigen following incubation with the peptide at various concentrations. Peptide competitively inhibited binding of NS19-9 MAb in dose dependent manner (concentration range of 10 nM to 5 mM), suggesting that it sterically interferes with MAb recognition of carbohydrate antigen. The IC_{50} value of the peptide was calculated at 700 μM. Control peptide DEVRPDLISTEE failed to compete with MAb NS 19-9 binding to SA-Le[a] at the same concentration range. Similarly, peptide DLWDWVVGKPAG did not block the binding of MAb BR15-A specific for carbohydrate LeY antigen (36) indicating that the inhibitory effects of the peptide #4 are due to specific recognition by MAb NS 19-9.

Acute inflammation model in vivo. Early steps in the recruitment of neutrophils to the site of inflammation depend upon E-selectin-mediated interaction and SA-LeX. An interaction of E-selectin with carbohydrate ligand, SA-Le[a], is not relevant for adhesion of neutrophils, since SA-Le[a] is not expressed on polymorphonuclear (PMN) surface. Nevertheless, because SA-Le[a] binds to E-selectin, we tested whether administration of a SA-Le[a] mimicking molecule would diminish the influx of neutrophils into chemically irritated peritoneum *in vivo.* To assess the bioactivity of the peptide #4 DLWDWVVGKPAG mimicking SA-Le[a] carbohydrate, the Zymosan was administered ip into mice followed 3 h later by an iv injection of peptide (1 mg/mouse). Neutrophils were harvested by peritoneal lavage and enumerated 1 h later. Thirty-percent of reduction in number of neutrophils in peritoneal lavage fluids was observed upon peptide treatment (Fig. 1A). Control experiments using the same dose of peptide DEVRPDLISTEE, which does not bind to NS-19-9 MAb, showed no decrease in neutrophil influx relative to PBS-injected mice. The mean values for experimental group and control animals were 1.2×10^7 (SD 0.51) and 1.7×10^7 (SD 0.49), respectively. The results were statistically significant ($P < 0.023$).

To confirm these results, MPO activity, which is an enzymatic marker for neutrophils, was measured spectrophotometrically as an absorbance rate in the homogenates of cells collected from peritoneal cavity (35). Thirty-percent of reduction of enzymatic activity was observed in parallel with decreased neutrophil num-

bers assessed by total neutrophil count (Fig. 1B). The mean values were calculated as 0.19 (SD 0.087) and 0.264 (SD 0.097) for experimental group and control animals, respectively. The results were statistically significant ($P < 0.043$) and strongly suggest that the reduction in enzyme activity is due to blocking in neutrophil recruitment in mice treated with peptide mimicking SA-Le^a.

DISCUSSION

The structural relevance of the distinct motifs observed in the families of peptides is unclear. They may indicate that MAb could tolerate a variety of amino acid substitutions within the peptide sequence that allow retaining structural mimicry and functional specificity of the peptides. Alternatively, peptides based on different consensus sequences isolated with the same MAb can mimic different structural topographies of the SA-Le^a carbohydrate and thus represent nonoverlapping surfaces of cognate antigen. Although, the peptide DLWDWVVGKPAG shows relatively low-affinity binding with the antibody, a strong binding signal was observed on the Western blot when the peptide sequence is expressed in the context of thioredoxin. This might be a reflection of conformational constraints imposed by thioredoxin on the inserted peptide compared with the structural properties of the peptide in solution or in solid phase. The ability to select of low affinity peptides during the panning procedure might also reflect a multivalent binding interactions between MAb and thioredoxin molecules expressed on the bacterial surface. Yet, the peptide inhibition of MAb NS19-9 binding to carbohydrate antigen implies that the sequence DLWDWVVGKPAG acts as structural mimic of SA-Le^a and represents a solvent-accessible epitope for MAb binding site.

The observed reduction of neutrophil recruitment into peritoneal cavity suggests that SA-Le^a peptidomimetic may block E-selectin interaction with of SA-LeX ligand expressed on neutrophils. The results also suggest that the shared molecular topography between isomeric structures SA-Le^a and SA-LeX might be important for E-selectin recognition and *in vivo* function. Sialic acid and fucose residues are in virtually identical positions in space providing similar topographies on each molecules for E-selectin recognition (22). The structure-function relationship study and conformational analysis have led to the rational development of SA-LeX mimetic, which are comparable or better than the natural ligand as inhibitors of E-selectin. SA-LeX mimetic identified in computer-based pharmacophore search, using energetically preferred conformation of a saccharide has identified glycyrrihizin, which fucosylated derivative was effective selectin antagonist and blocked neutrophil recruitment in mouse model (37).

The high IC_{50} value obtained from *in vitro* competition assay for peptide DLWDWVVGKPAG used in our study may not be relevant to the *in vivo* situation where blocking effect was clearly observed. One mg dose of this peptide yielded 30% of specific reduction of neutrophil recruitment into peritoneal cavity as measured by neutrophil count and MPO activity. Similar level of protection against tissue injury paralleled reduction of neutrophil recruitment obtained with SA-LeX oligosaccharide in rat lung injury model (38). In these experiments 35% of specific reduction of neutrophil mobilization was achieved using monovalent, tetra and/or pentasaccharides administered i.v. Similar results were obtained with anti-selectin antibodies, or selectin-Ig chimeric protein (39) and peptides derived from the conserved region of the lectin recognition domain of selectins (25, 26).

The family of high affinity peptides isolated from the combinatorial library using E-selectin-IgG fusion protein was previously characterized and peptide DITWDQLWDLMK showed binding affinity to E-selectin at nM level (31). However, the peptide did not demonstrate blocking ability of E-selectin and SA-LeX interaction and did not require calcium for binding. The peptide contains an identical sequence (Gln-Leu-Trp-Asp) to the first four amino acid residues of PSGL-1 glycoprotein core, which represents co-receptor for both P- and E-selectin (40). Consequently, this peptide does not define a carbohydrate mimotope, but perhaps represents a protein binding epitope for the selectins. Conversely, the peptide DLWDWVVGKPAG identified in our study inhibits interaction of MAb with the cognate antigen, suggesting that peptide binding occur at or near the carbohydrate-binding site of the MAb. Surprisingly, the comparison of the amino acid sequence of these two peptides revealed identical Leu-Trp-Asp sequence. This suggests that tripeptide may provide topological similarity between SA-Le^a and other E-selectin ligands and it is partially responsible for blocking of neutrophil influx in our study. However, Gln-Leu-Trp-Asp sequence is not sufficient to mimic SA-LeX topography recognized by E-selectin and to accommodate into of E-selectin binding site, since peptide DITWDQLWDLMK was not able to block interaction of E-selectin and SA-LeX. Thus, the amino acid residues surrounding this tetrapeptide contributing to the specificity and the affinity of the interaction between E-selectin and the peptide do not provide structural features of carbohydrate ligand. In contrast, the amino acids flanking the shared tripeptide Leu-Trp-Asp in DLWDWVVGKPAG sequence must be responsible for structural SA-Le^a carbohydrate mimicry.

Our results demonstrate that using a combinatorial approach based on functional equivalence of the chemically dissimilar molecules sharing common surface topology instead of derivatized parental structures using chemoenzymatic approach is effective in develop-

ing antagonists of physiologically important molecular interactions. We have demonstrated that peptide mimicking carbohydrate determinant retained conformational properties of cognate carbohydrate structure. The peptide mimic is an antagonist of the carbohydrate ligand functional interaction *in vivo* as shown using inhibition of leukocyte trafficking to the site of inflammation.

ACKNOWLEDGMENTS

We thank Hildegund Ertl for support and encouragement. The studies were supported by Grant DAMD17-96-1-6232 from Department of the Army Medical Research and Material Command (M.B.T.) and in part from NIH Grant AI45133 (T.K.E.).

REFERENCES

1. Valadon, P., Nussbaum, G., Boyd, L. F., Margulies, D. H., and Scharff, M. D. (1996) *J. Mol. Biol.* **261,** 11–22.
2. Bianchi, E., Folgori, A., Wallace, A., Nicotra, M., Acali, S., Phalipon, A., Barbato, G., Bazzo, R., Cortese, R., Felici, F., and Pessi, A. (1995) *J. Mol. Biol.* **247,** 154–160.
3. Harris, S. L., Craig, L., Mehroke, J. S., Rashed, M., Zwick, M. B., Kenar, K., Toone, E. J., Greenspan, N., Auzanneau, F. I., Marino-Alberanas, J. R., Pinto, B. M., and Scott, J. K. (1997) *J. Biol. Chem.* **94,** 2454–2459.
4. Kaur, K. J., Khurana, S., and Salunke, D. M. (1997) *J. Biol. Chem.* **272,** 5539–5543.
5. Oldenburg, K. R., Loganathan D., Goldstein, I. J., Schultz, P. G., and Gallop, M. A. (1992) *J. Biol. Chem.* **89,** 5393–5397.
6. Scott, J. K., Loganathan, D., Easley, R. B., Gong, X., and Goldstein, I. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5398–5402.
7. Taki, T., Ishikawa, D., Hamasaki, H., and Hando, S. (1997) *FEBS Lett.* **418,** 219–223.
8. Hoess, R., Brinkmann, U., Handel, R., and Pastan, I. (1993) *Gene* **128,** 43–49.
9. Lou Q., and Pastan I. (1999) *J. Pept. Res.* **53,** 252–260.
10. Kieber-Emmons, T., Luo, P., Qiu, J., Chang, T. Y., O, I., Blaszczyk-Thurin, M., and Steplewski, Z. (1999) *Nature Biotechnol.* **17,** 660–665.
11. Vertesy, L., Oeding, V., Bender, R., Zepf, K., and Nesemann, G. (1984) *Eur. J. Biochem.* **141,** 505–512.
12. Froude, J., Gibofsky, A., Buskirk, D. R., Khanna, A., and Zabriskie, J. B. (1989) *Curr. Topics Microbiol. Immunol.* **145,** 5–26.
13. Moran, A. P., Prendergast, M. M., and Appelmelk, B. J. (1996) *FEMS Immunol. Med. Microbiol.* **16,** 105–115.
14. Misasi, R., Dionisi, S., Farilla, L., Carabba, B., Lenti, L., Di Mario, U., and Dotta, F. (1997) *Diabetes Metab. Rev.* **13,** 163–179.
15. Bevilacqua, M. P., and Nelson, R. M. (1993) *J. Clin. Invest.* **91,** 379–387.
16. Kansas, G. S. (1996) *Blood* **88,** 3259–3287.
17. Lasky, L. A. (1995) *Annu. Rev. Biochem.* **64,** 113–139.
18. Zimmerman, G. A., McIntyre, T. M., and Prescott, S. M. (1996) *J. Clin. Invest.* **98,** 1699–1702.
19. Varki, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7390–7397.
20. Hakomori, S. (1996) *Cancer Res. Metab.* **56,** 5309–5318.
21. Magnani, J. L., Brockhaus, M., Smith, D. F., Ginsburg, V., Blaszczyk, M., Mitchell, K. F., Steplewski, Z., and Koprowski, H. (1981) *Science* **212,** 55–56.
22. Berg, E. L., Robinson, M. K., Mansson, O., Butcher, E. C., and Magnani, J. L. (1991) *J. Biol. Chem.* **266,** 14869–14872.
23. Watson, S. R., Fennie, C., and Lasky, L. A. (1991) *Nature (London)* **349,** 164–167.
24. Welply, J. K., Abbas, S. Z., Scudder, P., Keene, J. L., Broschat, K., Casnocha, S., Gorka, C., Steiniger, C., Howard, S. C., and Schmuke, J. J. (1994) *Glycobiology* **4,** 259–265.
25. Geng, J. G., Haevner, G. A., and McEver, R. P. (1993) *J. Biol. Chem.* **267,** 19846–19853.
26. Briggs, J. B., Oda, Y., Gilbert, J. H., Schaeffer, M. E., and Macher, B. A. (1995) *Glycoconjugate J.* **12,** 535.
27. O'Connell, D., Koenig, A., Jennings, S., Hicke, B., Han, H. L., Fitzwater, T., Chang, Y. F., Varki, N., Parma, D., and Varki, A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5883–5588.
28. Nelson, R. M., Cecconi, O., Roberts, W. G., Aruffo, A., Linhardt, R. J., and Bevilaqua, M. P. (1993) *Blood* **82,** 3253–3258.
29. Cecconi, O., Nelson, R. M., Roberts, W. G., Hanasaki, K., Mannori, G., Schultz, C., Ulich, T. R., Aruffo, A., and Bevilaqua, M. P. (1994) *J. Biol. Chem.* **269,** 15060–15066.
30. Ley, K., Linnemann, G., Meinen, M., Stoolman, L. M., and Gaethengs, P. (1993) *Blood* **81,** 177–185.
31. Martens, C. L., Cwirla, S. E., Lee, R. Y., Whitehorn, E., Chen, E. Y., Bakker, A., Martin, E. L., Wagstrom, C., Gopalan, P., Smith, C. W., Tate, E., Koller, K. J., Schatz, P. J., Dower, W. J., and Barret, R. W. (1995) *J. Biol. Chem.* **270,** 21129–21136.
32. O, I., Kieber-Emmons, T., Otvos, L., and Blaszczyk-Thurin, M. (1999) *Ann. N.Y. Acad. Sci.*, in press. [abstract]
33. Lu, Z., Murray, K. S., Van Cleave, V., LaVallie, E. R., Stahl, M. L., and McCoy, J. M. (1995) *Biotechnology* **13,** 366–372.
34. Towbin, H., Stachelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
35. Bradley, P. P., Priebat, D. A., Christensen, R. D., and Rothstein, G. (1982) *J. Invest. Dermatol.* **78,** 206–209.
36. Rodeck, U., Herlyn, M., Leander, K., Borlinghaus, P., and Koprowski, H. (1987) *Hybridoma* **6,** 389–401.
37. Rao, B. N., Anderson, M. B., Musser, J. H., Gilbert, J. H., Schaefer, M. E., Foxall, C., and Brandley, B. K. (1994) *J. Biol. Chem.* **269,** 19663–19666.
38. Mulligan, M. S., Lowe, J. B., Larsen, R. D., Paulson, J., Zheng, Z. L., DeFrees, S., Maemura, K., Fukuda, M., and Ward, P. A. (1993) *J. Exp. Med.* **178,** 623–631.
39. Mulligan, M. S., Watson, S. R., Fennie, C., and Ward, P. A. (1993) *J. Immunol.* **151,** 6410–6417.
40. Moore, K. L., Stults, N. L., Diaz, S., Smith, D. F., Cummings, R. D., Varki, A., and McEver, R. P. (1992) *J. Cell. Biol.* **118,** 445–446.